

Analytical Supercritical Fluid Extraction: Current Trends and Future Vistas

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Current Trends

Supercritical fluids have been used as unique solvents in a number of analytical techniques (1), such as nuclear magnetic resonance spectroscopy (2), thin-layer chromatography (TLC) (3), and field flow fractionation (4). The analytical chemist, however, normally associates the prefix "supercritical fluid" with chromatographic or extraction methodologies that have been extensively developed within the past 15 years. The "renaissance" of supercritical fluid chromatography (SFC) occurred in the mid-1970s, largely as a result of improvements in injection and pumping devices, enhanced column efficiencies, and the refinement of transport mechanisms to deliver the separated solutes to modified gas (GC) and liquid chromatographic (LC) detectors (5). By contrast, supercritical fluid extraction (SFE), despite a long history as a physicochemical phenomenon (6) and a recent plethora of applications in chemical engineering (7), has developed as an analytical technique only since the mid-1980s and is presently in an "evolutionary" state.

Despite the early pioneering research of Stahl in coupling SFE with TLC (8), activity in "analytical" SFE remained limited until it was introduced as a pendant technique coupled to SFC. However, this tandem methodology severely limited the sample size that could be extracted with these dense fluids and placed a dual burden on the analyst to optimize both SFE and SFC simultaneously. Because the future of SFC was unknown at the time, commercial instrument manufacturers opted to scale extraction cells to be compatible with the fluid volume and delivery rate capabilities of the syringe pumps used in SFC. Indeed, many of the early extraction cells consisted of modified LC guard cartridges, which were eventually replaced by cell designs that could be "finger-tightened" to withstand extraction pressures up to 10 000 psi.

The development of analytical SFE has also been somewhat hampered by a lack of theoretical guidelines that can be applied to the diverse array of sample types and matrixes encountered by the analyst. Nevertheless, a number of theoretical concepts,

ranging from the "solubility parameter theory" (9) to the "hot ball" kinetic model (10), have been applied to optimize SFE conditions. The limitations of such theoretical models, however, become apparent in the actual practice of SFE, as demonstrated by the difference in analyte solubility in a supercritical fluid and its extractability from a specific matrix. For the moment, therefore, experimental optimization of the extraction conditions appears to be the surest way of attaining high analyte recoveries.

The current practice of analytical SFE is divided between "off-line" and "on-line" methods, despite their common physicochemical basis. Such definitions refer to the mechanism of conducting the extraction. The on-line methods are usually combinations of SFE with ancillary techniques such as GC, SFC, LC, or gel permeation chromatography (GPC). Off-line SFE, the current method in vogue, offers more flexibility with respect to extracting different sample sizes and types, as well as in the choice of the final analytical method. Nevertheless, the selection of an SFE method should be based on the problem facing the analyst.

To date, carbon dioxide has been by far the supercritical fluid of choice for analytical SFE (11). The selection of CO₂ was initially based on its widespread use in SFC, its low critical temperature (31°C), and the high degree of nonideality that the gas exhibits even at relatively low levels of compression. Moreover, recent concerns over environmental pollution, the exposure of laboratory workers to noxious solvents, and the disposal costs associated with organic solvents have placed analytical SFE using CO₂ in a different light. The suggested use of such an innocuous solvent as a substitute for organic solvents has been met with skepticism from some scientists. However, supercritical carbon dioxide (SC-CO₂) has proved to be a convenient solvent, whether used as a medium for conducting "Soxhlet-type" extractions of lipid material in various sample matrixes (12) or for extracting ultralow level trace analytes, such as pesticide or drug moieties, from an assortment of sample types (13).

In general, SC-CO₂ mimics the solvent behavior of nonpolar to moderately polar solvents (14). The dissolution power of a supercritical fluid is exponentially proportional to its fluid density; therefore, rapid and exhaustive extractions are best

handled by conducting the extractions at high pressures. Extraction selectivity for a particular species in SFE is achieved by operating at a lower extraction pressure, but usually at the expense of solute solubility. However, with respect to trace analysis, many analytes, even extremely polar moieties, can be readily dissolved in SC-CO₂ at the ppm or ppb level. Neat SC-CO₂ should not be perceived as a "magic solvent," capable of extracting only the desired target analytes. Coextractives will frequently dissolve in supercritical fluid solvents, just as in liquid solvent extraction. Selectivity can be enhanced for an analyte, or a particular class of analytes (15), by incorporating adsorbents, either *in situ* or after the initial extraction stage (16).

SFE has been clearly demonstrated to efficiently extract many pesticides and specific drug moieties. Much of the evidence supporting this conclusion has come from extractions performed on spiked sample matrixes (17). However, recent reports of results obtained on samples containing incurred residues (18, 19) confirm the efficacy of SC-CO₂ as an extraction solvent for these analytes. Polar analytes, such as antibiotics, remain a challenge to the analyst because of their lower solubility in SC-CO₂ and their partitioning equilibria, which favor an aqueous medium. However, a number of extraction options remain to be explored with respect to these polar solutes, including the addition of low levels of organic cosolvents or special additives to the supercritical fluid. An impressive range of solubility enhancements for polar solutes in these additive/cosolvent-supercritical fluid systems was recorded (20), and the amount of cosolvent required is still considerably below the volumes required in conventional liquid-based extractions.

The coupled or on-line SFE methodologies mentioned previously appear to have special applications and problems connected with their use. On-line SFE can truly be regarded as a "micro SFE" technique, because the sample sizes used are frequently small (mg level) to avoid solute overload on microbore chromatographic columns. These coupled methods have proved to be of particular value in characterizing small samples such as single seeds, fibers, and live insects. However, considerable skill is required of the analyst to produce uniform and consistent extraction results. On-line SFE tends to be prone to contamination, and the diminutive scale of the technique raises questions about sample uniformity. In addition, procedures for concentrating extracts, such as cryofocusing or sorbent trapping, which are frequently used in on-line SFE/SFC methods, are still poorly understood and are capable of introducing bias in the analytical result.

Current trends in analytical SFE are diverse and worthy of comment. The recent introduction of instrumentation capable of performing extractions on larger and more representative samples is one current trend. As a result, instrumentation manufacturers have had to consider the design of supercritical fluid delivery systems with respect to higher fluid flow rates and extraction pressures. Likewise, the development of multi-sample extractors (21, 22) for the simultaneous processing of large numbers of samples has further catalyzed the creation of new instrumentation. One specialized application of analytical SFE, the determination of fat levels in food products, requires instru-

mentation capable of producing very high extraction pressures and fluid flow rates. Optimization of the extraction conditions for removing lipid moieties (23) results in the efficient and rapid removal of fats by SC-CO₂ in 15–20 min (24).

The purity level of extraction fluid such as CO₂ has always been suspect, particularly when SFE in either the off- or on-line mode is used in conjunction with ultrasensitive, element-specific detectors. To cite a specific example, the use of off-line SFE in conjunction with electron capture detection, using impure grades of CO₂, will limit the detectability of organochlorine pesticides below the sub-ppm level. Commercial gas manufacturers have recently responded to such needs by producing ultrahigh purity CO₂ in which the total impurity level is in the range of 10 ppt as determined by an electron capture detector (25, 26). These grades of extraction fluids can be expensive for use of exhaustive, high pressure SFE with large samples, suggesting that sorbent-based gas purification systems attached to the extractor modules will need to be developed in the near future. Hence, even industrial grades of CO₂ will have a place in analytical SFE, particularly in the routine determination of the fat content of samples.

The influence of the sample matrix on SFE results was noted by one of the authors (27). Control of sample matrix effects is critical in SFE to limit coextractives, moderate the influence of moisture, and improve the efficiency of the extraction. Recent studies have shown that the addition of both inert and active sorbents to the sample matrix can improve the efficiency of SFE (28). Extractions from difficult sample matrixes, such as soils, can be improved by adding various cosolvents or by using other supercritical fluids, such as nitrous oxide (29, 30).

Future Vistas

What does the future hold for analytical SFE? We believe that the optimal SFE system has yet to be created. Extraction systems need to be developed that offer the flexibility of operating at both higher and lower pressure ranges. Current instrumentation has reached the 10 000 psi level, but theory suggests that many useful extractions can be conducted at higher temperatures and pressures. These conditions will certainly increase the potential molecular weight range of nonthermally labile solutes that can be extracted, but instrumentation must be constructed that is capable of maintaining the proper fluid densities at elevated temperatures. Likewise, SFE is an excellent technique for examining volatile components because the extractions can be conducted at relatively low temperatures and in a nonoxidative environment. These target analytes can best be extracted at pressures that are very low by conventional SFE standards. However, to date most extraction systems offer limited control at the lower extraction pressures required for analysis of volatiles. Certainly, SFE is a viable alternative to headspace techniques, which depend on thermal energy to volatilize analytes; hence, the authors can envisage a bright future for SFE in sensory analysis problems.

Postextraction fractionation will play an increasingly important role in the future of SFE. As alluded to earlier, target

analytes such as pesticides and drugs can be selectively fractionated from unwanted coextractives by using selective sorbents packed in the extraction vessel or in individual vessels downstream from the extraction stage. Such crude fractionations may be viewed as a simple form of normal-phase chromatography when SC-CO₂ is used as the eluant. As such, the analyst should be able to apply LC principles to design the most appropriate supercritical fractionation system. Extraction of unwanted materials by SFE, a form of "inverse" SFE, falls within the context of supercritical fluid fractionations. This form of sample cleanup was already demonstrated on an engineering scale (31) and offers the possibility of isolating analytes from interfering components.

The advantages and disadvantages of coupling SFE with other analytical techniques have already been noted. Several detection and identification schemes coupling mass spectrometry (MS) and Fourier-transform infrared spectroscopy (FTIR) were published (32, 33), but thus far have not found widespread use by the analytical community. Undoubtedly, further research will result in the adoption of some of these hybrid techniques by the analytical chemist. To date, most of the coupled technologies have used rather sophisticated instruments or detectors with SFE. However, a relatively untouched area is the coupling of SFE with simple chemical tests or techniques. One tandem method that was recently explored is the coupling of SFE with immunoassays (33) for the rapid assessment of pesticide contamination in meat products. Such a method offers the possibility of implementation on-site, at a processing plant or inspection station. The method can be made to work with a "pumpless" SFE system and introduces only benign SC-CO₂ and water into the environment. Such simplified SFE systems, along with field portable instrumentation, constitute a wave of the future in analytical SFE.

Conclusion

In concluding our overview of analytical SFE, we should like to make several comments about its implementation in regulatory chemistry protocols. No single analytical technique can hope to solve the diversity of sample preparation problems confronting the analyst; however, analytical SFE will eventually take its rightful place among other sample preparation methods. Successful implementation of SFE will require that analysts expand their horizons and trade some of their conventional tools, such as volumetric flasks and beakers, for pressure gauges and extraction cells. At the same time, the proponents of SFE should attempt to integrate the technique into established analytical protocols, thereby facilitating an easy transition for the bench analyst.

Interest in SFE remains high among such agencies as the Agricultural Research Service (ARS), U.S. Food and Drug Administration (FDA), Food Safety and Inspection Service (FSIS), U.S. Environmental Protection Agency (EPA), Federal Grain and Inspection Service (FGIS), Agricultural Marketing Service (AMS), and some state monitoring agencies. A round robin type of study on sediments and dust has been undertaken by the National Institute of Science and Technology (NIST)

and EPA laboratories in the hope of ascertaining the reproducibility of the technique between laboratories. ARS and FDA laboratories now have multi-sample extraction equipment, and commercial counterparts are available. SFE in the laboratories of ARS and FDA was shown to yield both complete and reproducible extractions for pesticide residues down to the 0.5 ppb level. A rough analysis of the savings afforded by using SC-CO₂ as the extraction solvent in place of conventional organic solvents used in the PAM procedures indicates a cost savings of 94–97% (34, 35). This does not include the disposal costs of the organic solvents. However, governmental and industrial laboratories must make their needs known to instrumentation companies if the technique is to remain viable.

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